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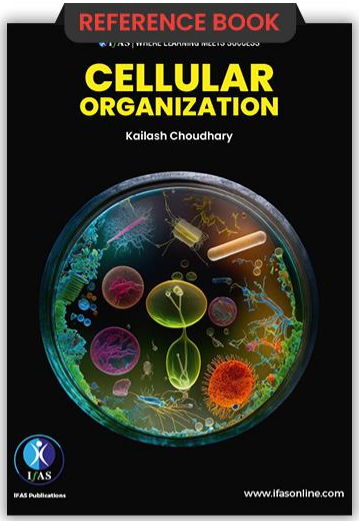
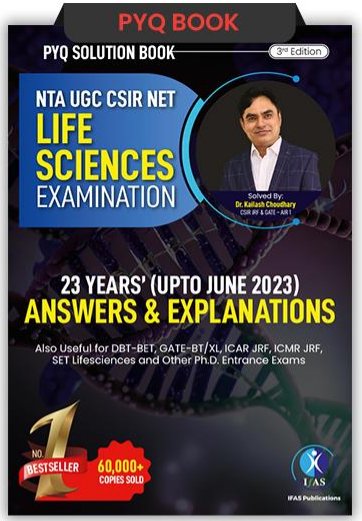
CSIR NET – Life Science

Unit 2: Cellular Organization

L9 Cytosolic Protein Targeting

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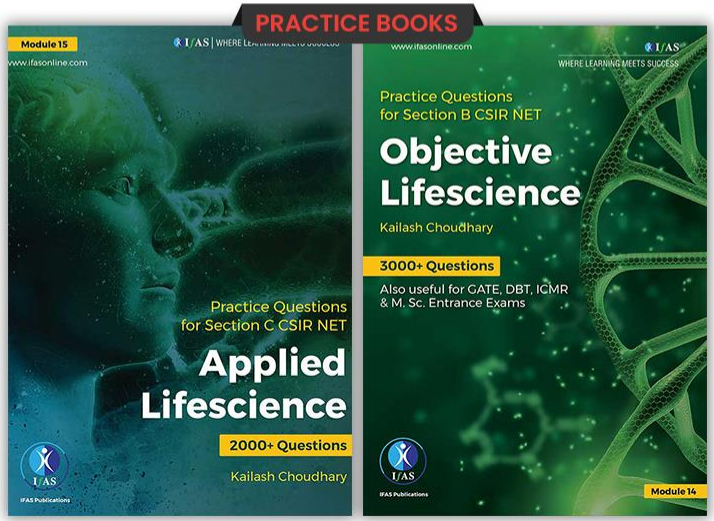

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Points to be covered in this Lecture

- Cytosolic Protein Targeting
- Peroxisomes
- Plastids
- Mitochondria

Cytosolic Protein Targeting

Protein synthesis completed in cytosol

Protein targeting

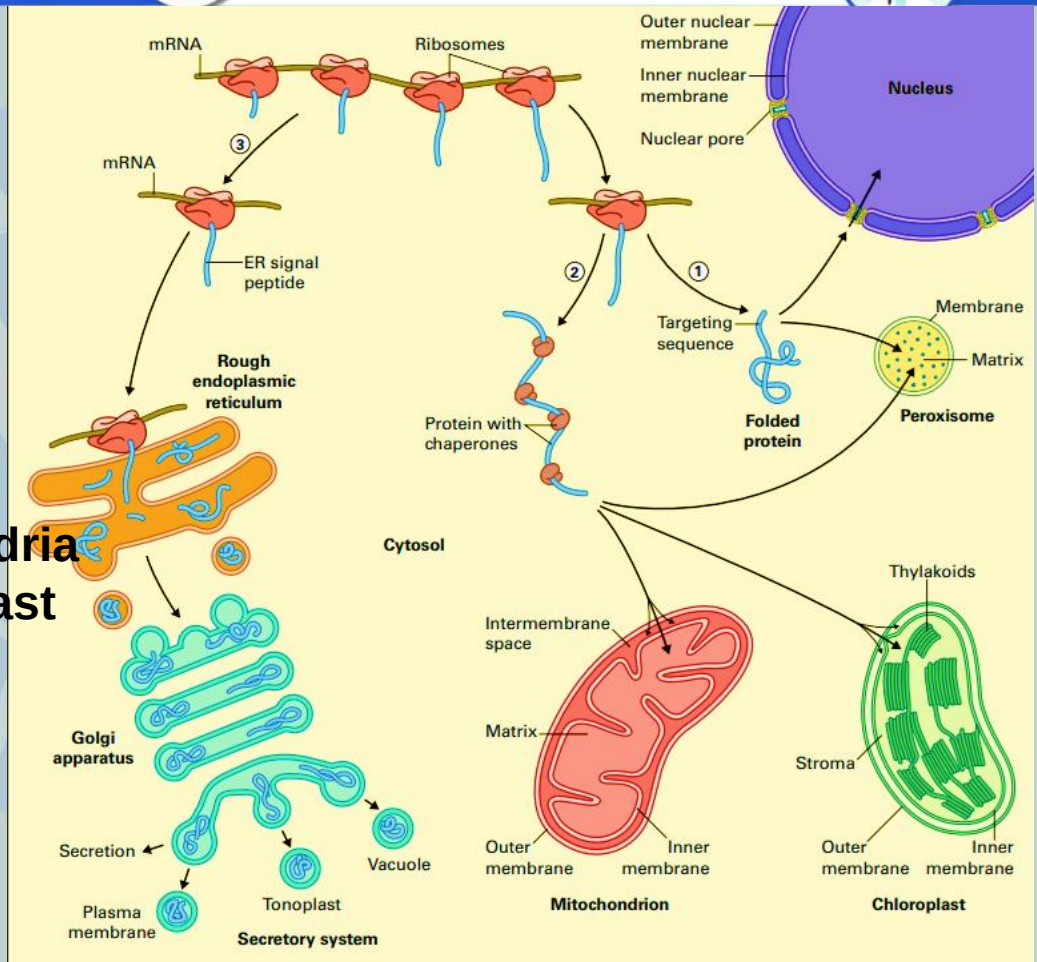
Folded

Nucleus

Peroxysosme

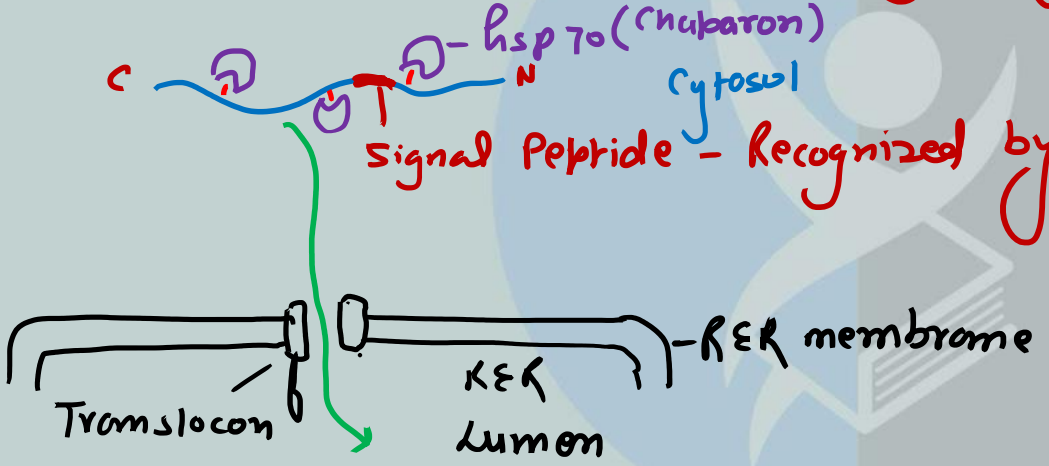
Unfolded

- **Mitochondria**
- **Chloroplast**



Note → in yeast some **Secretory protein** also undergoes

Post translational Targeting



Recognized by TRC 40 in yeast helps in post Translational Targeting

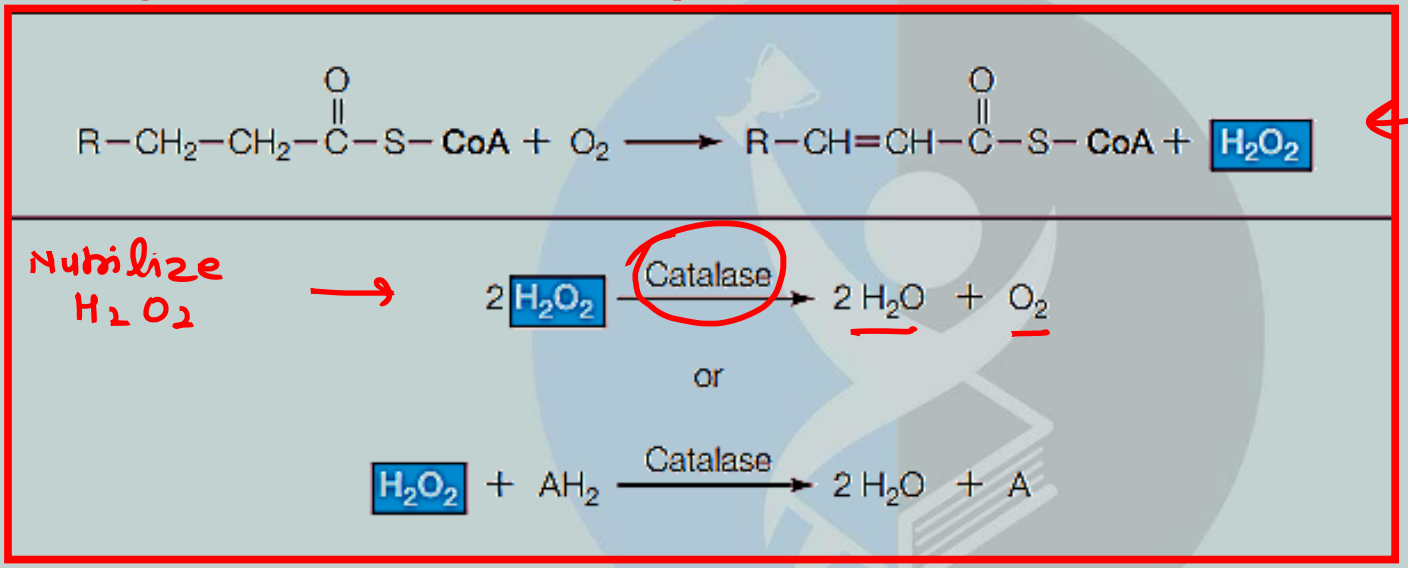
Peroxisomes

- Single-membrane-enclosed organelles.
- Contain enzymes involved in a variety of metabolic reactions, including - energy metabolism

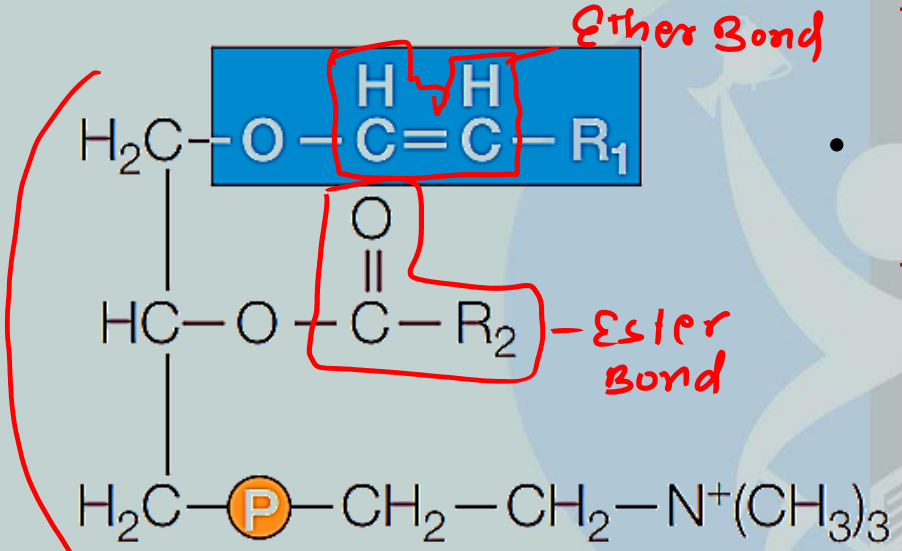
Functions of Peroxisomes

- Oxidation reactions of organic compounds
- Catabolism of very long chain fatty acids, branched chain fatty acids, D-amino acids and polyamines.
- Biosynthesis of plasmalogens and ether phospholipids
- Metabolism of ureides and reactive oxygen species (H_2O_2)
- Beta oxidation of fatty acid and glyoxylate cycle in plants
- Marker enzyme is catalase
- X-linked adrenoleukodystrophy, Zellweger syndrome (ZS) and Refsum disease are diseases related with peroxisomes.

Fatty acid oxidation in peroxisomes



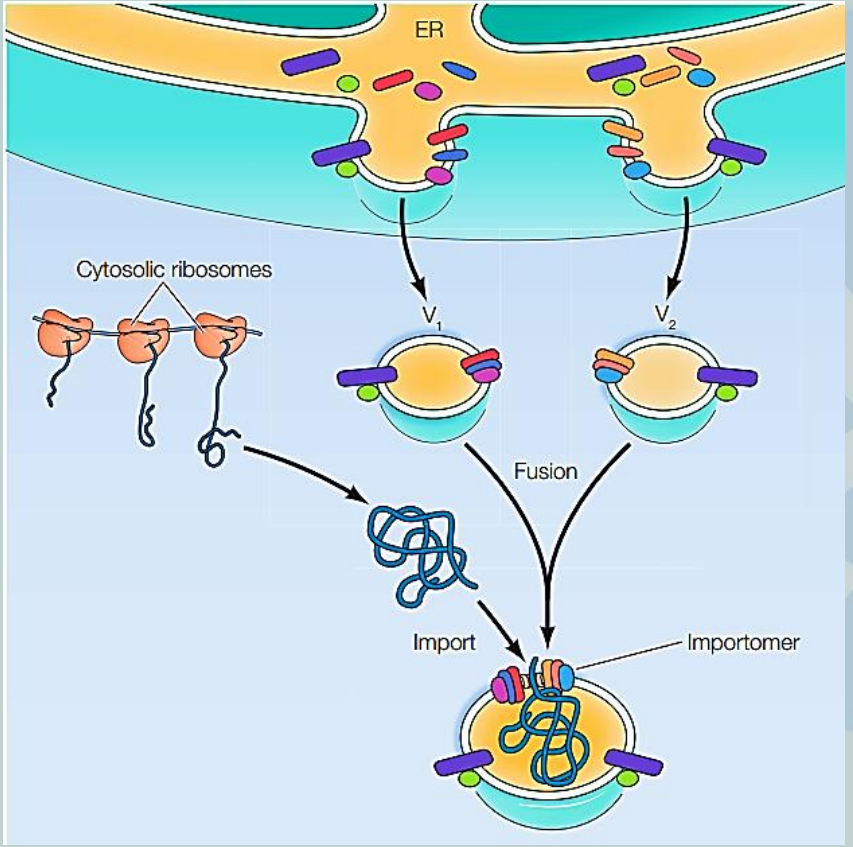
Structure of a plasmalogen



- The plasmalogen shown is analogous to phosphatidylcholine.
- However, one of the fatty acid chains is joined to glycerol by an ether, rather than by an ester, bond.

Plasmalogens are important membrane components in some tissues, particularly heart and brain, although they are absent in others

Assembly of peroxisomes



- Peroxisomal transmembrane proteins are derived from the ER.
- Two different types of vesicles (designated V1 and V2) carrying distinct transmembrane proteins fuse to form a functional peroxisome.
- The combination of these different proteins forms the machinery (importomer) through which internal peroxisome proteins synthesized on cytosolic ribosomes can be imported

- Peroxisomes do not have their own genomes and all their proteins are synthesized from the nuclear genome
- Peroxisomal proteins are synthesized on free ribosomes and then imported into peroxisomes as completed polypeptide chains.
- However, some membrane proteins are transferred to peroxisomes from the endoplasmic reticulum. – Through vesicular fusion.
- The proteins of peroxisomes are typical eukaryotic proteins

Import of peroxisomal matrix proteins

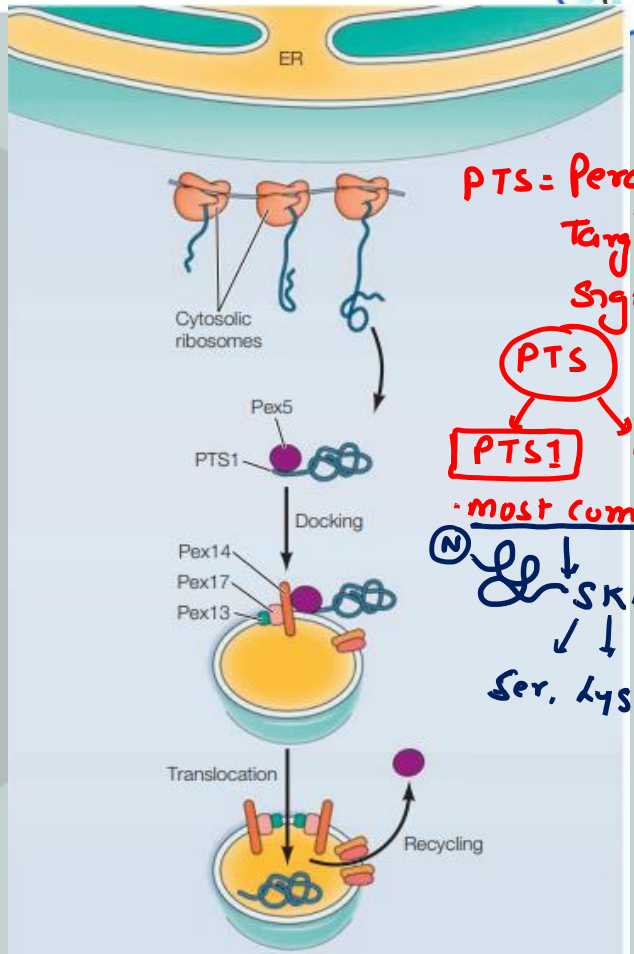
1. Peroxisomal matrix proteins containing the targeting signal PTS1

2. recognized by the cytosolic receptor Pex5.

3. The Pex5/cargo complex then binds to a docking complex (Pex13, Pex14, and Pex17) on the peroxisome membrane.

4. Pex5 and Pex14 form a pore in the membrane and the cargo protein is translocated into the peroxisome.

5. Pex5 is then recycled to the cytosol.



* if mutation in PTS-1 → Pex 5 unable to binds with Peroxysomal protein
  if mutation in Pex-5

if Pex-7 mutated / PTS-2 mutated
↓
Receptor of PTS-2
↓
Adenoleukodestrophy

↓
Peroxysomal protein fails to Target in Peroxysome
↓
Peroxysome fails to perform its Function
↓
Zellweger Syndrome

Plastids → present in plants & Absent in Animal & fungi

All plastids contain the same genome as chloroplasts, but they differ in both structure and function.

- The different types of plastids are frequently classified according to the kinds of pigments they contain

1 • **Chloroplasts** - contain chlorophyll.

- **Chromoplasts** - lack chlorophyll but contain carotenoids
  - responsible for the yellow, orange, and red colors of some flowers and fruits

- **Leucoplasts** (nonpigmented plastids)- store a variety of energy sources in nonphotosynthetic tissues.
  - roots, tuber
- Leucoplasts (**Amyloplasts** and **elaioplasts**)

store starch store lipids

Plastids

Chloroplast

- Photosynthesis, photo-phosphorylatuob, photo-resipiration, terpene biosynthesis, lipid biosynthesis

Chromoplast

- Pigment synthesis and storage

Leucoplast

- **Amyloplasts:** for starch storage
- **Statoliths**: for detecting gravity - Root -> Promote Gravitropism
- **Elaioplasts:** for storing fat and oil
- **Proteinoplasts**: for storing and modifying protein

Etioplast

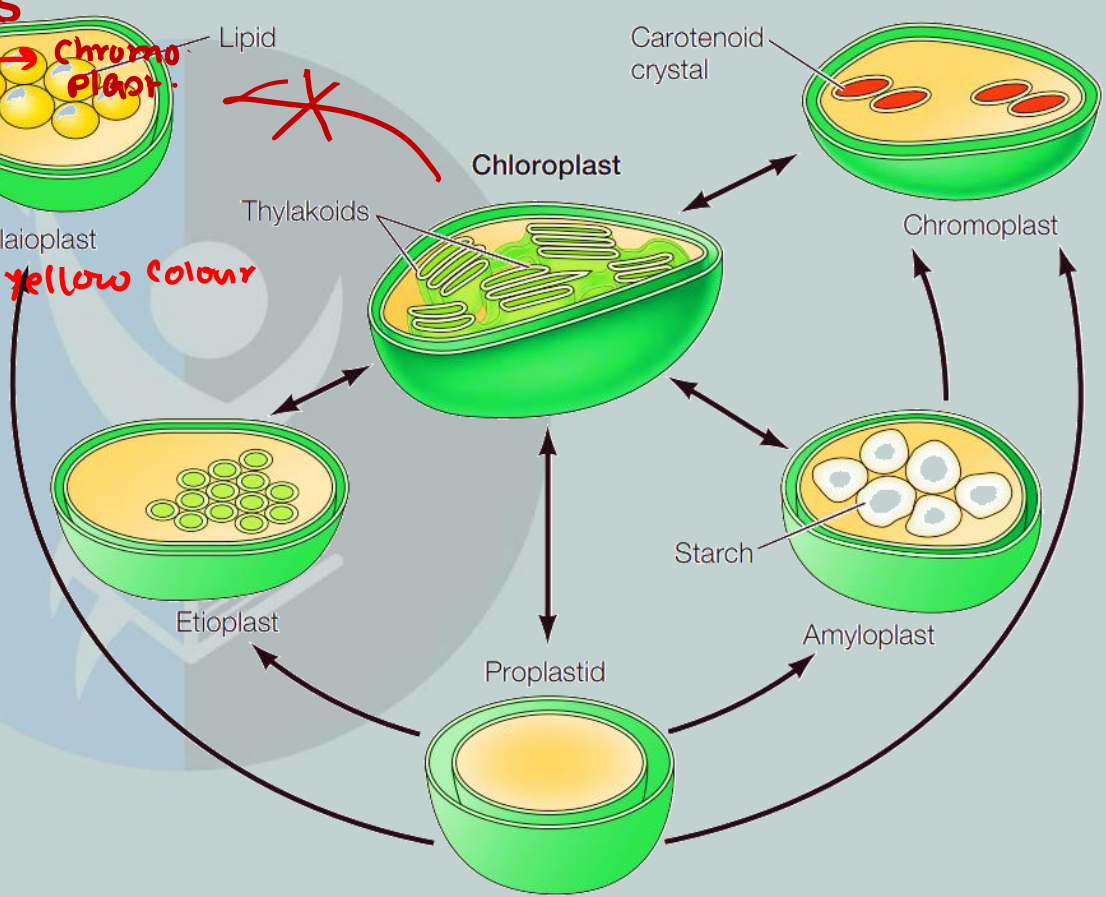
- Photosynthetically inactive chlorplast in absence of light.

Interconversions of plastids

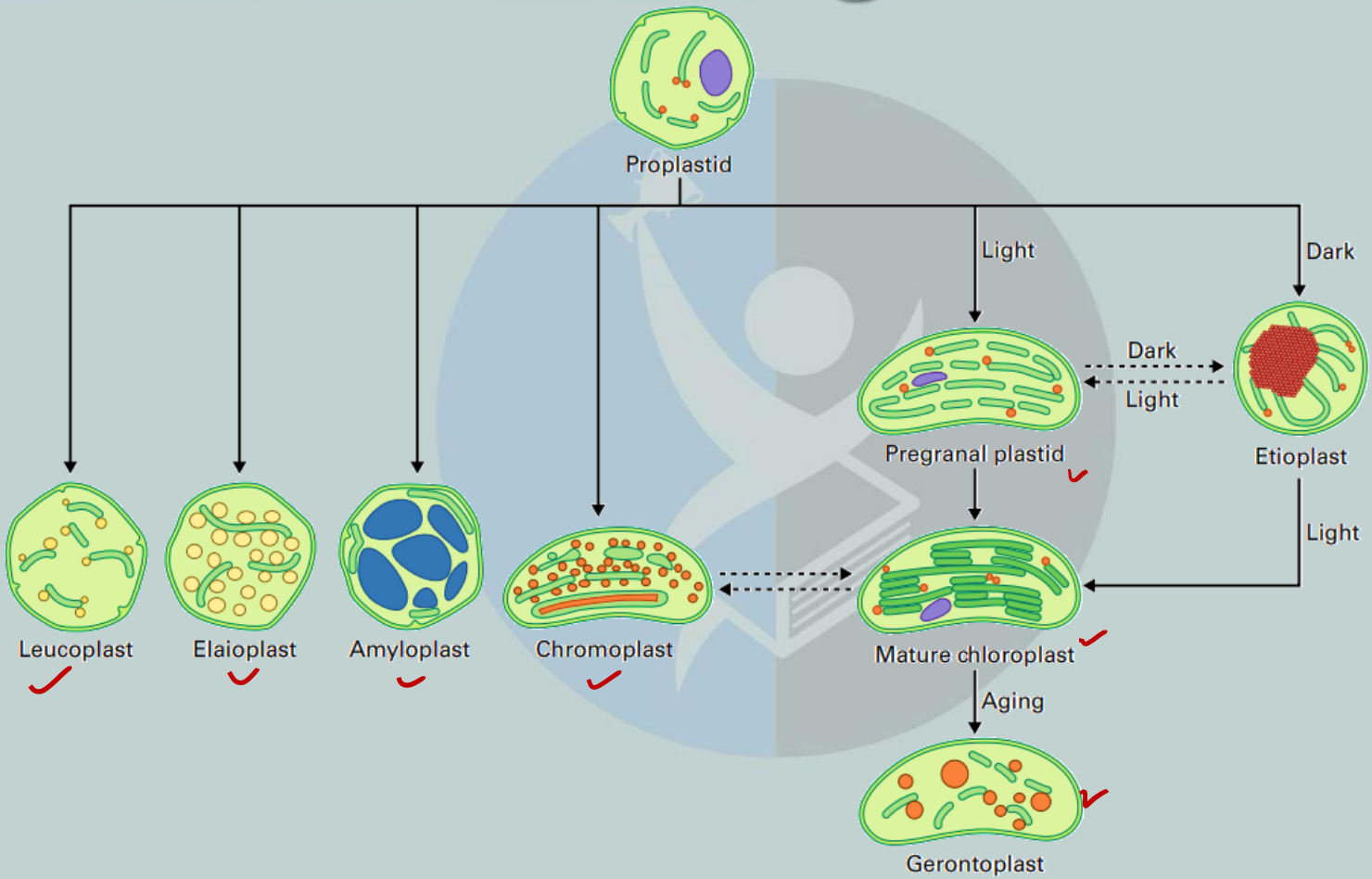
Leucoplast → (light) → Chloroplast → (light) → Chromoplast

Proplastids can develop into -

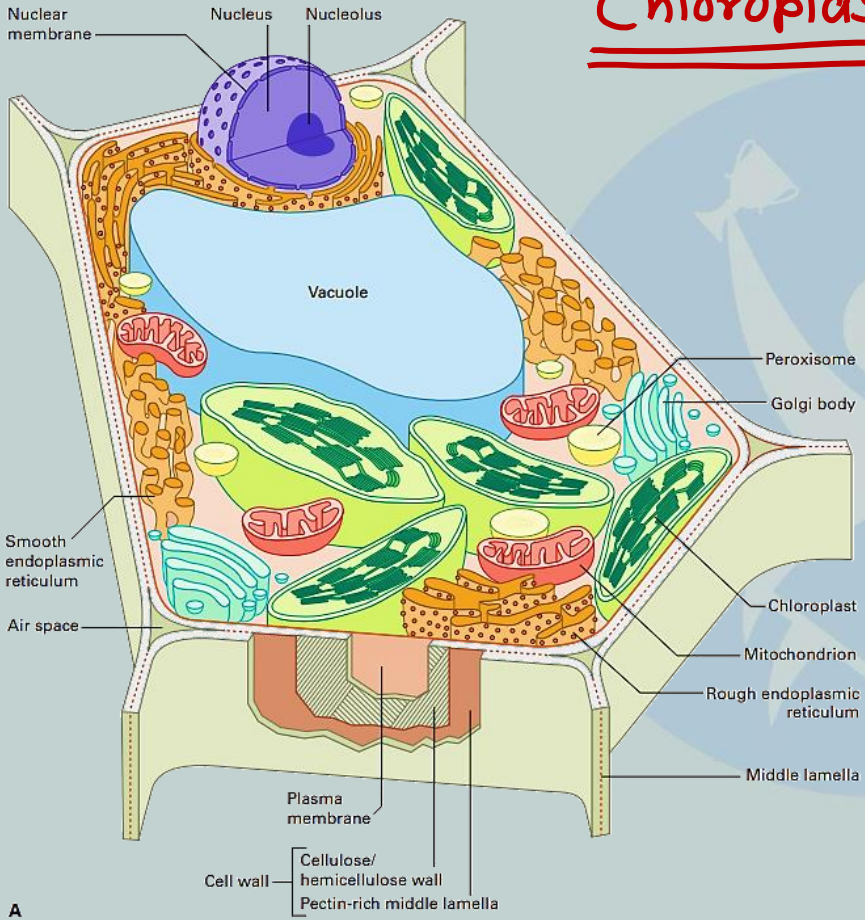
1. chloroplasts
2. chromoplasts, → xanthophyll → yellow colour
3. etioplasts,
4. elaioplasts, and
5. amyloplasts.

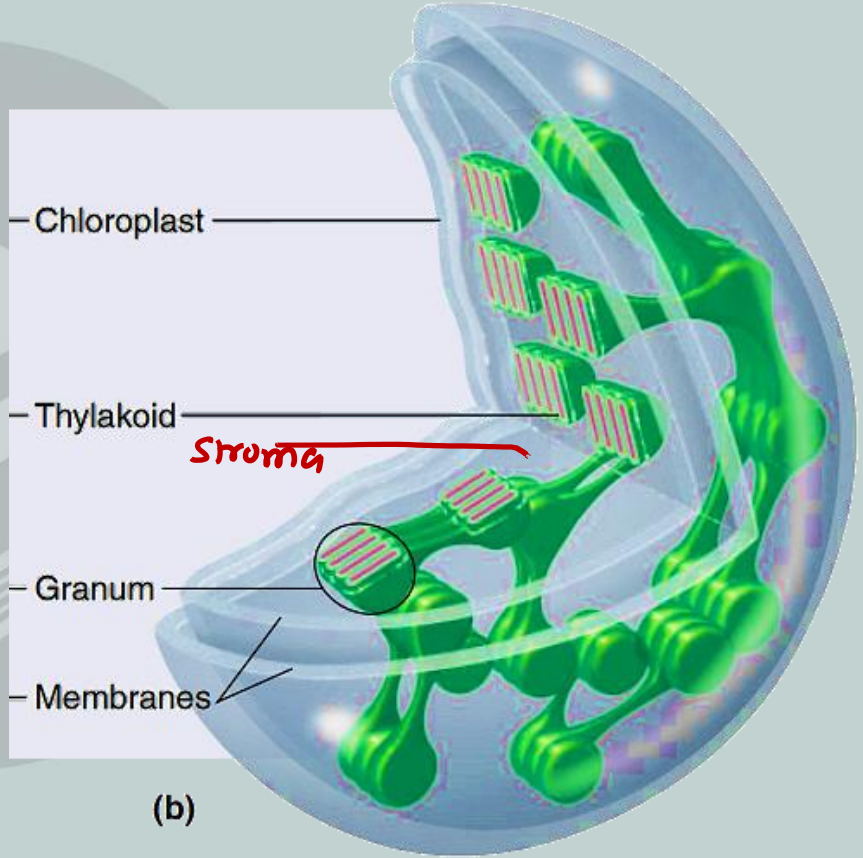


Different plastids can also convert to other types.

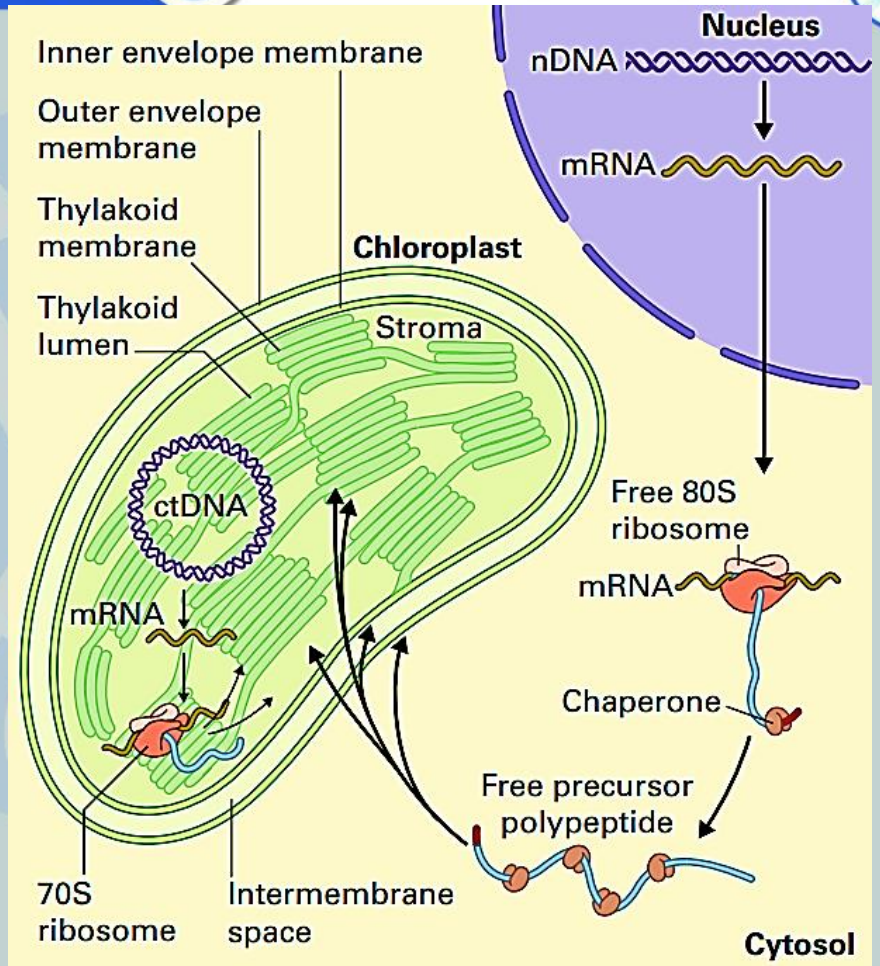

Proplastid
Light
Dark
Dark
Light
Pregranal plastid
Etioplast
Light
Leucoplast
Elaioplast
Amyloplast
Chromoplast
Mature chloroplast
Aging
Gerontoplast

Chloroplast



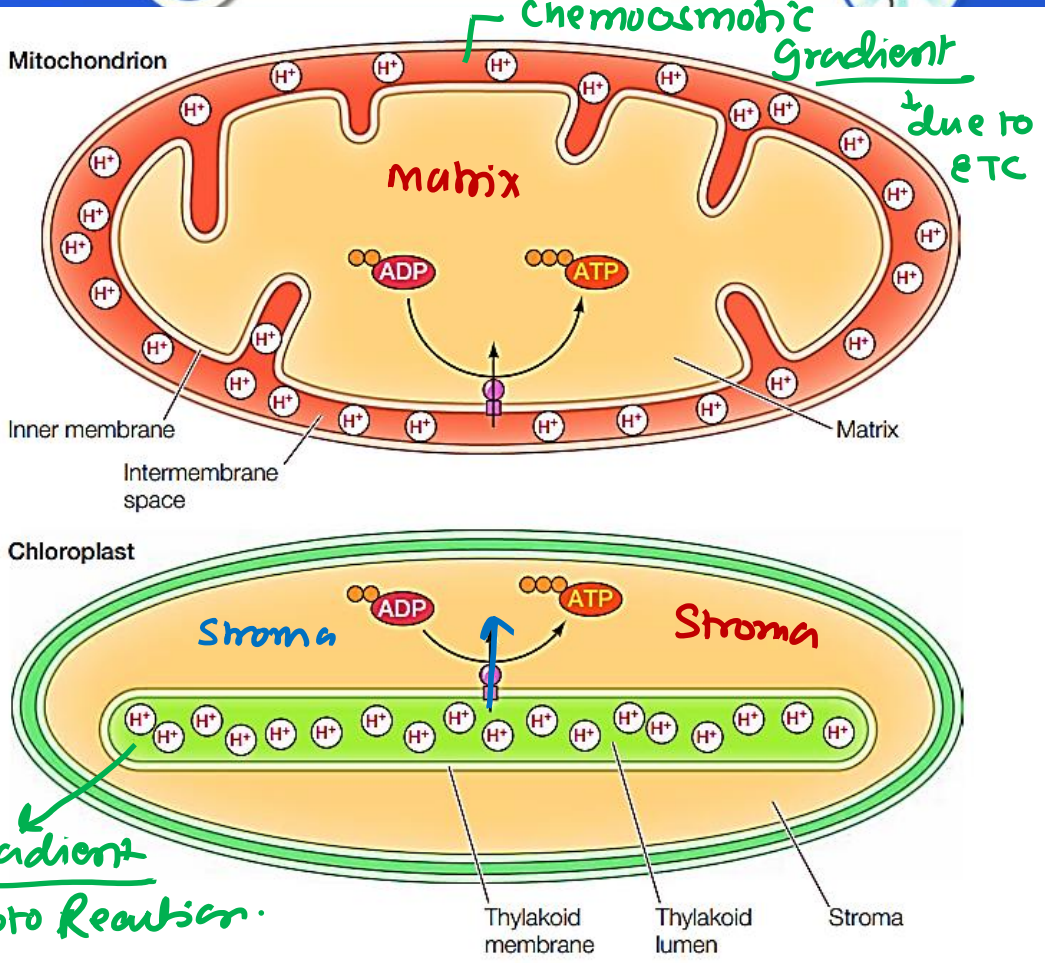


Chloroplast Protein Targeting.



Chemiosmotic generation of ATP in chloroplasts and mitochondria

- In mitochondria, electron transport generates a proton gradient across the inner membrane, which is then used to drive ATP synthesis in the matrix.
- In chloroplasts, the proton gradient is generated across the thylakoid membrane and used to drive ATP synthesis in the stroma



2 Genes Encoded by Chloroplast DNA

Function	Number of genes
Gene expression	
rRNAs (23S, 16S, 5S)	3
tRNAs	30
Ribosomal proteins	40
RNA polymerase subunits	4
Photosynthesis	
Photosystem I	8
Photosystem II	18
Cytochrome *bf* complex	8
ATP synthase	8
NADP reductase	11
Metabolism	11
Protein folding and membrane insertion	14

Chloroplast genomes

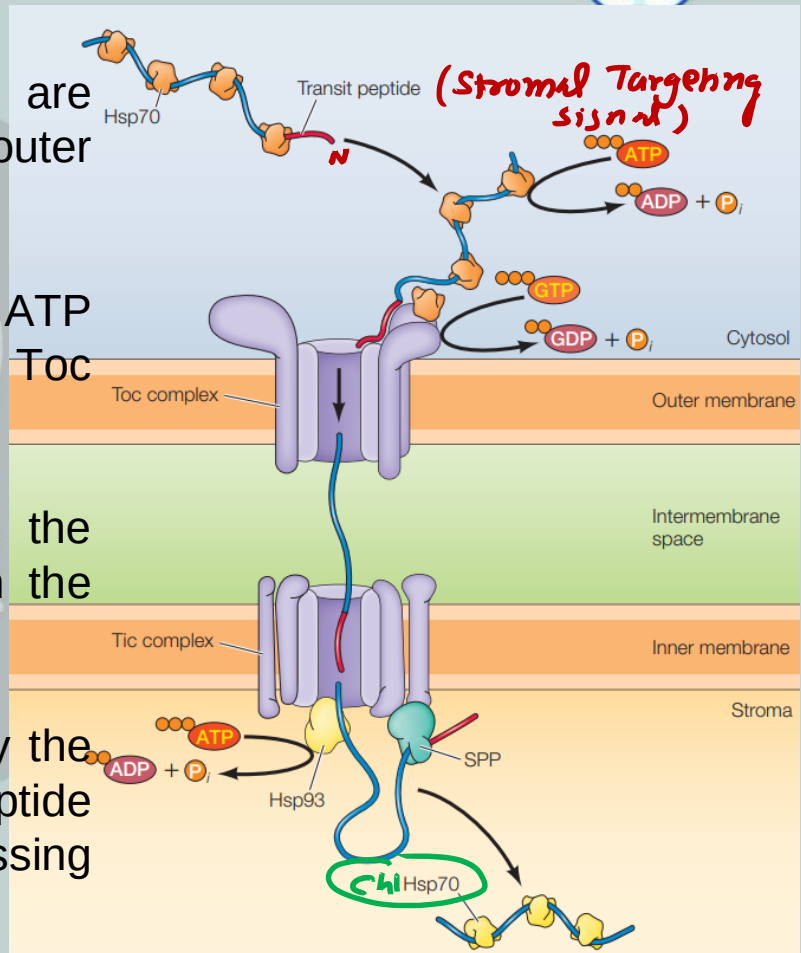
- Contain their own genetic system
- Evolutionary origins from photosynthetic bacteria.
- Circular DNA molecules present in multiple copies per organelle
- However, chloroplast genomes are larger and more complex than those of mitochondria, ranging from 100–200 kb and containing approximately 150 genes.

Most chloroplast proteins are targeted for import to chloroplasts by transit peptides → Present Toward N Terminus

- About 3000 or 95% of chloroplast proteins are still encoded by nuclear genes
- Rest of proteins are synthesized on cytosolic ribosomes and then imported into chloroplasts (Post translational targeting).

Import of proteins into the chloroplast

- Proteins with **N-terminal transit peptides** are targeted to the Toc complex in the chloroplast outer membrane.
- Passage through the outer membrane requires ATP hydrolysis by Hsp70 and hydrolysis of GTP by Toc proteins.
- Once through the chloroplast outer membrane, the transit peptide is passed to the Tic complex in the inner membrane.
- The protein is drawn through the Tic complex by the action of an Hsp93 chaperone and the transit peptide is removed by the chloroplast stromal processing peptidase (SPP).



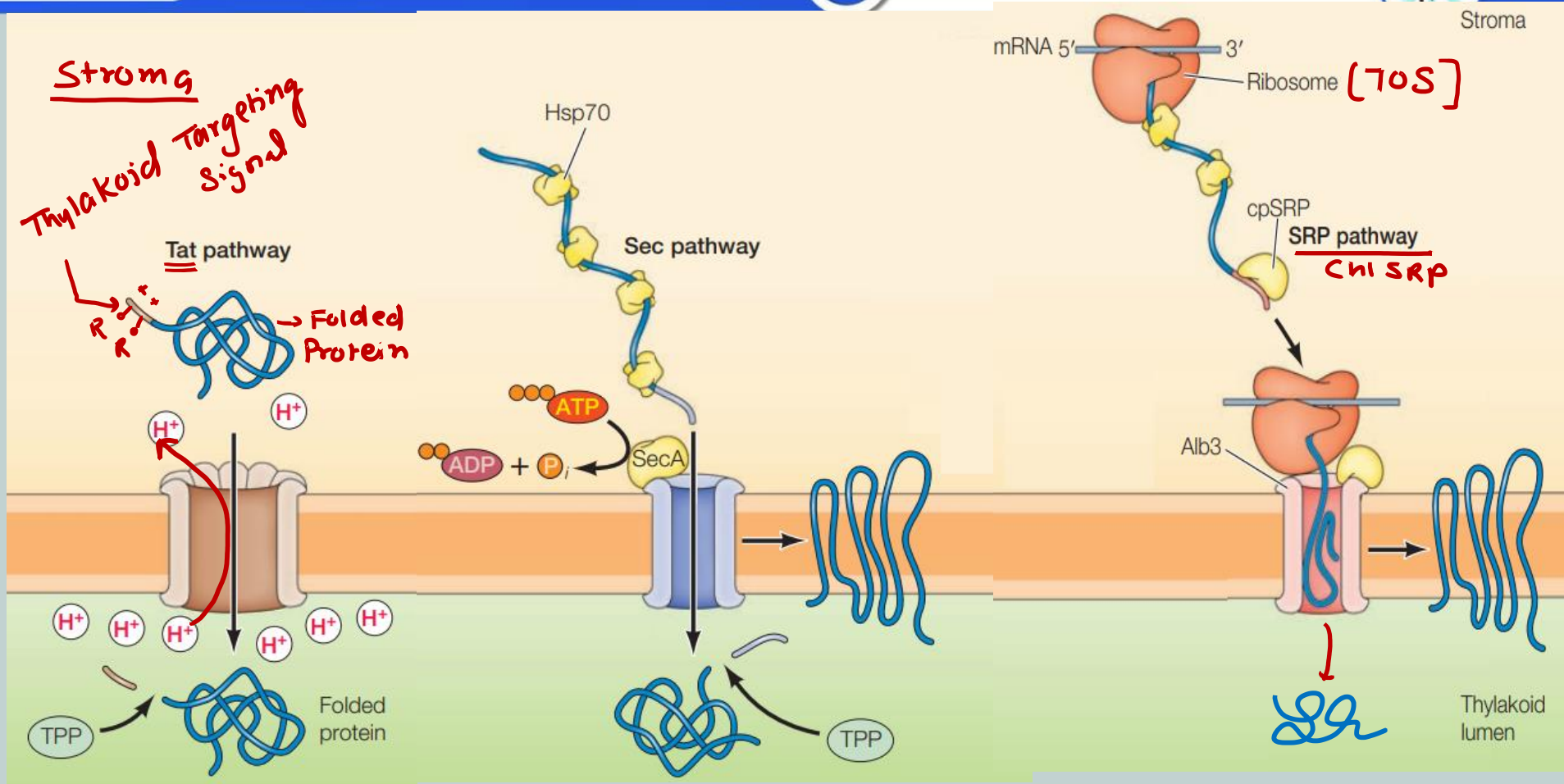
Some Toc proteins bind and hydrolyze GTP, providing an additional source of energy for translocation

Translocase of the chloroplast outer member (the Toc complex)

Translocase of the chloroplast inner member (the Tic complex)

Stromal processing peptidase (SPP)

Multiple pathways target proteins for translocation across the thylakoid membrane.


Stroma
Thylakoid Targeting Signal
Tat pathway
Folded Protein
H+
TPP
Folded protein
Hsp70
Sec pathway
ATP
ADP + Pi
SecA
TPP
mRNA 5′
3′
Ribosome (70S)
cpSRP
SRP pathway
Chl SRP
Alb3
Thylakoid lumen

Import of proteins into the thylakoid lumen and membrane

- In the **twin-arginine translocation (Tat) pathway**, - thylakoid signal sequence containing two arginines near the amino terminus targets fully folded proteins to the Tat translocon.
- Energy derived from the proton gradient across the thylakoid membrane.
- In the lumen, the thylakoid signal sequences are cleaved by a thylakoid processing protease (TPP).

- **Sec pathway** - SecA protein recognizes a thylakoid signal sequence and targets the protein to the Sec translocon,
- energy derived from ATP hydrolysis to transfer the protein to the lumen.

- Some transmembrane proteins exit the Sec translocase laterally for incorporation into the thylakoid membrane.

SRP pathways

- Transmembrane proteins, which may be either imported into the stroma or synthesized by chloroplast ribosomes within the stroma,

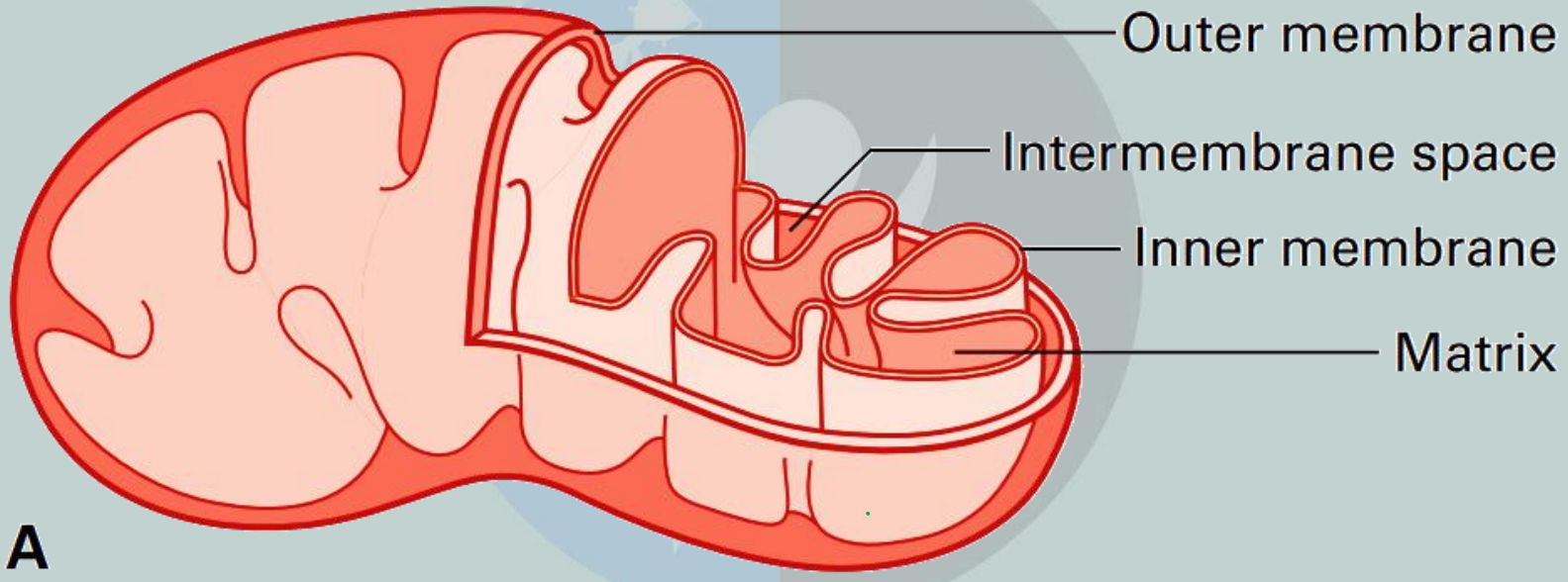
- Recognized by the chloroplast signal recognition particle (cpSRP) and inserted into the thylakoid membrane by the

- Chloroplast - maternal inheritance

Disease → Due to Chloroplast gene mutation

eg. • Variegated Leaf Pattern

Mitochondria



- play a critical role in the generation of metabolic energy in eukaryotic cells.
- ATP by the process of **oxidative phosphorylation**.
- **Porins** on outer mitochondrial membrane, form channels that allow the free diffusion of molecules smaller than about 1000 daltons.
- The genomes of **living organisms** that are most similar to the mitochondrial genome are those of free-living **α-proteobacteria**, which code for approximately 7000 proteins.

- An intracellular parasite like the α-proteobacterium Rickettsia prowazekii has a smaller genome, about 830 protein-coding genes.
- Like mitochondria, **Rickettsia prowazekii** is able to reproduce only within eukaryotic cells,
- but unlike mitochondria it still transcribes and translates most of its own genes.
- DNA molecules like those of bacteria, which are present in multiple copies per organelle.
- They vary considerably in size between different species

- The genomes of human and most other animal mitochondria - about 16 kb,
- yeasts -approximately 80 kb
- plants - more than 200 kb.
- Larger mitochondrial genomes are composed predominantly of noncoding sequences and do not appear to contain significantly more genetic information.
- For example, the mitochondrial genome of the plant Arabidopsis thaliana is approximately 370 kb but it encodes only 31 proteins: just more than twice the number encoded by human mitochondrial DNA.

The human mitochondrial genome

- Most mitochondrial proteins are translated on free cytosolic ribosomes and imported into the organelle by specific targeting signals.
- The genome contains 13 protein coding sequences, which are designated as components of respiratory complexes I, III, IV, or V.
- contains genes for 16S and 12S rRNAs and for 22 tRNAs.
- The region of the genome designated "D loop" contains an origin of DNA replication and transcriptional promoter sequences

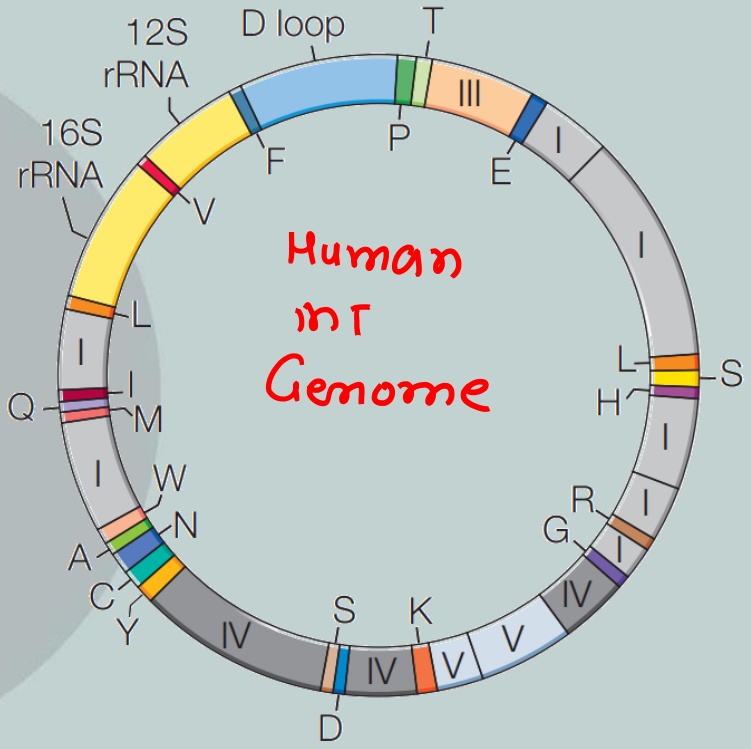
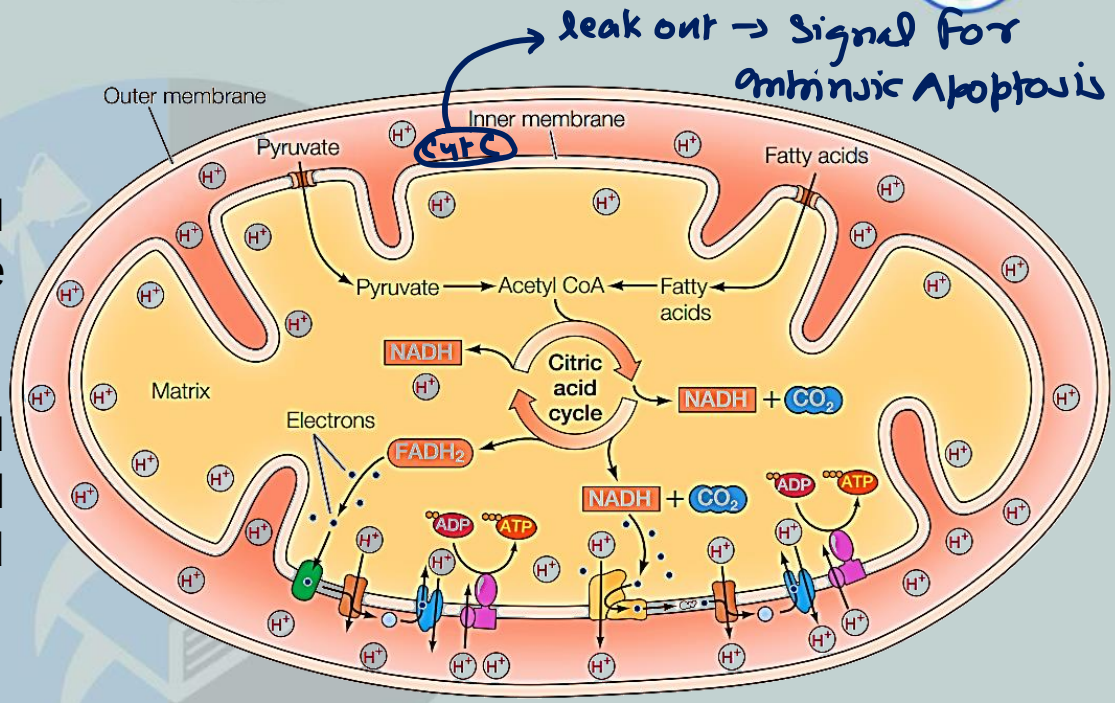


Table 13.1 Differences between the Universal and Mitochondrial Genetic Codes

Codon	Universal code	Human mitochondrial code
UGA	Stop	Trp
AGA	Arg	Stop
AGG	Arg	Stop
AUA	Ile	Met

Oxidative metabolism in mitochondria

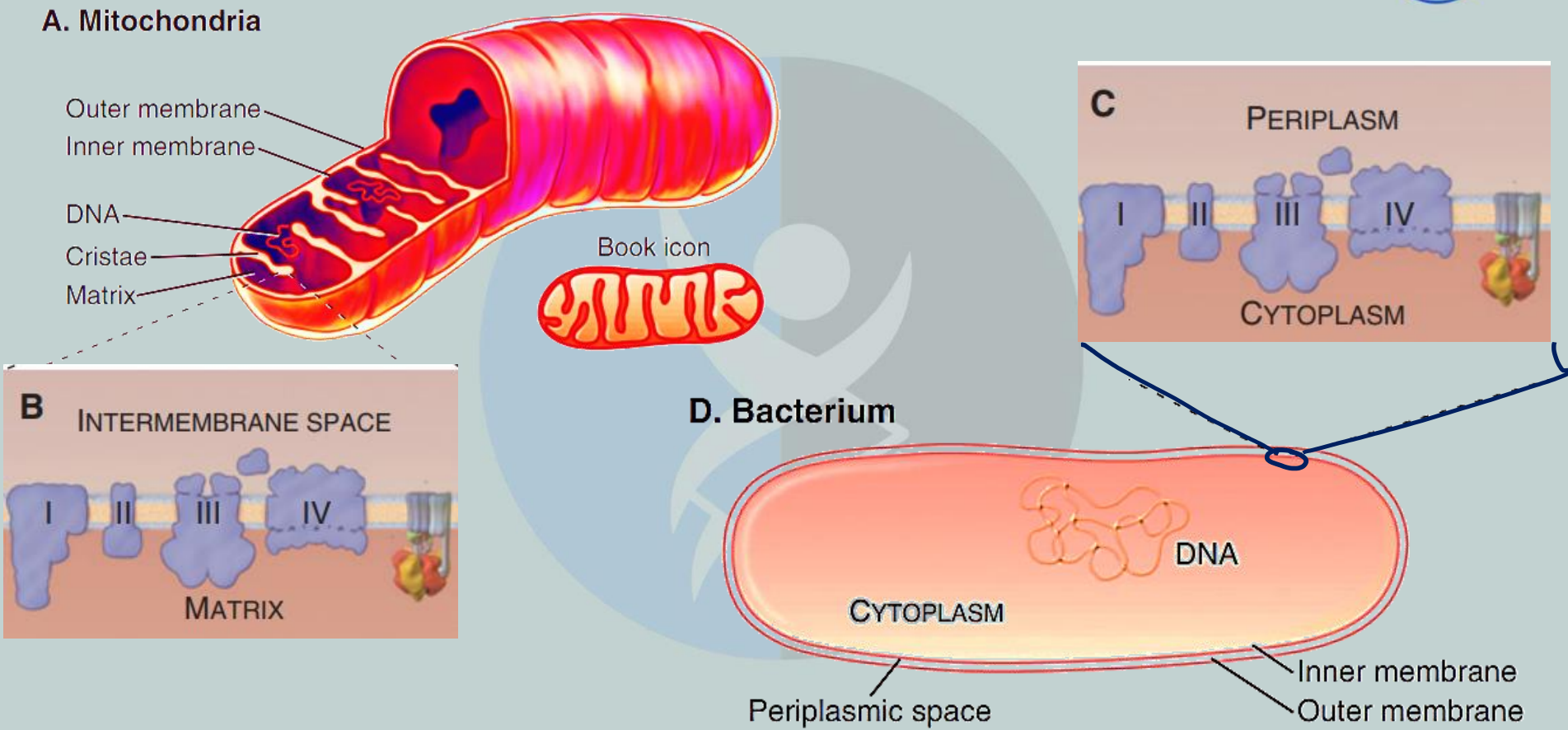
- The inner mitochondrial membrane is the site of oxidative phosphorylation.
- The integrity of the mitochondrial outer membrane plays a critical role in regulating programmed cell death



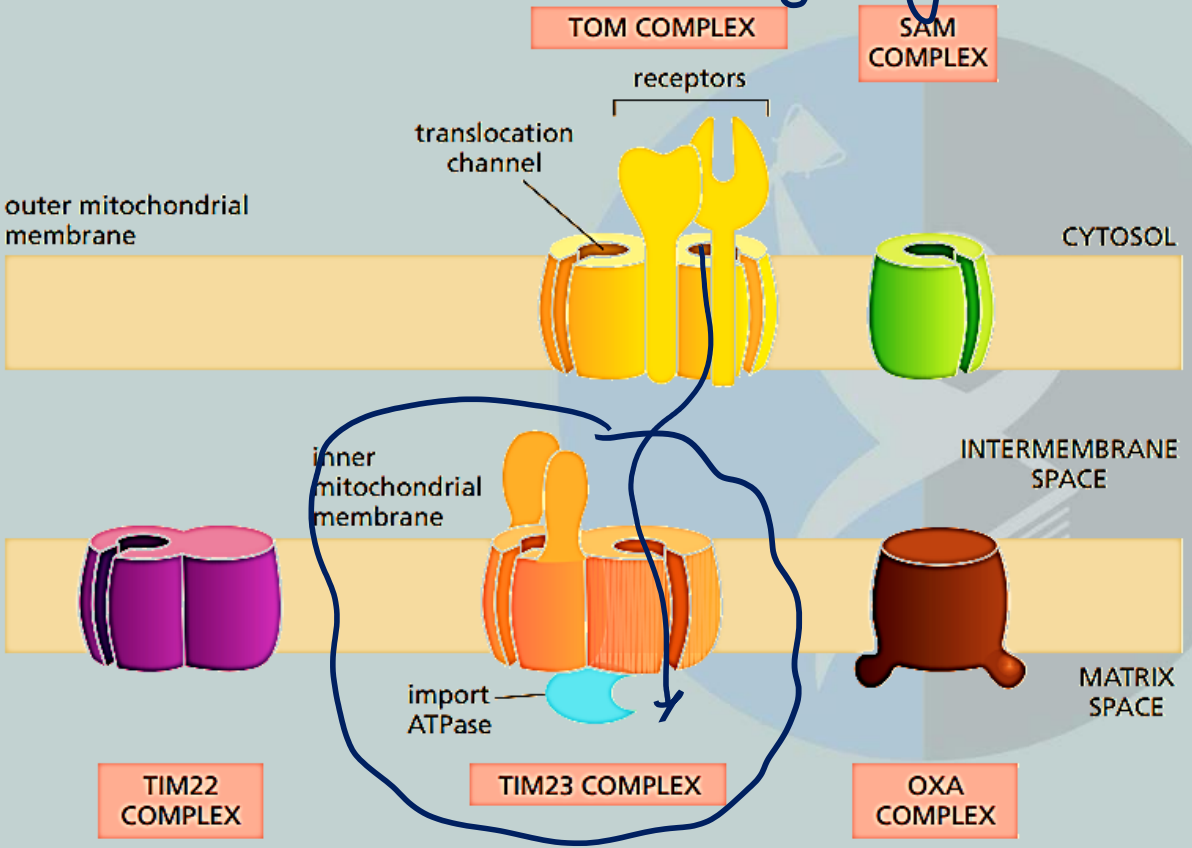
Intrinsic Apoptosis
· Cyt. C

mT

- Although their genomes encode only 13 proteins, mammalian mitochondria contain about 1500 different proteins that are encoded by the nuclear genome of the cell.


A. Mitochondria
Outer membrane
Inner membrane
DNA
Cristae
Matrix
Book icon
B
INTERMEMBRANE SPACE
I
II
III
IV
MATRIX
C
PERIPLASM
I
II
III
IV
CYTOPLASM
D. Bacterium
DNA
CYTOPLASM
Periplasmic space
Inner membrane
Outer membrane

Mitochondrial Protein Targeting.



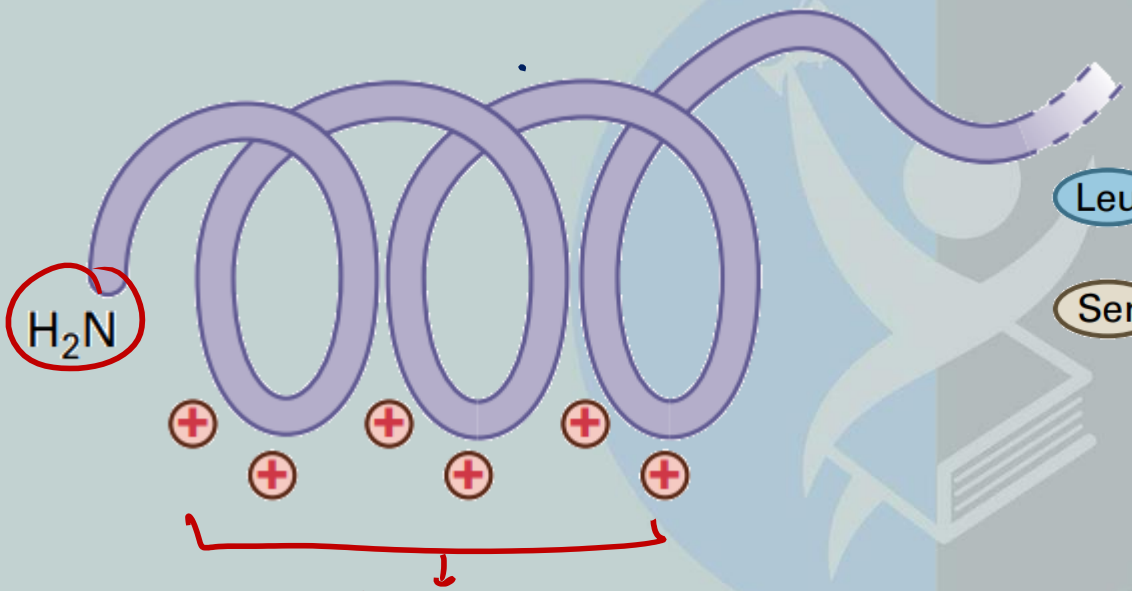
SAM = Sorting and Assembly Machinery;

OXA = cytochrome OXidase Activity;

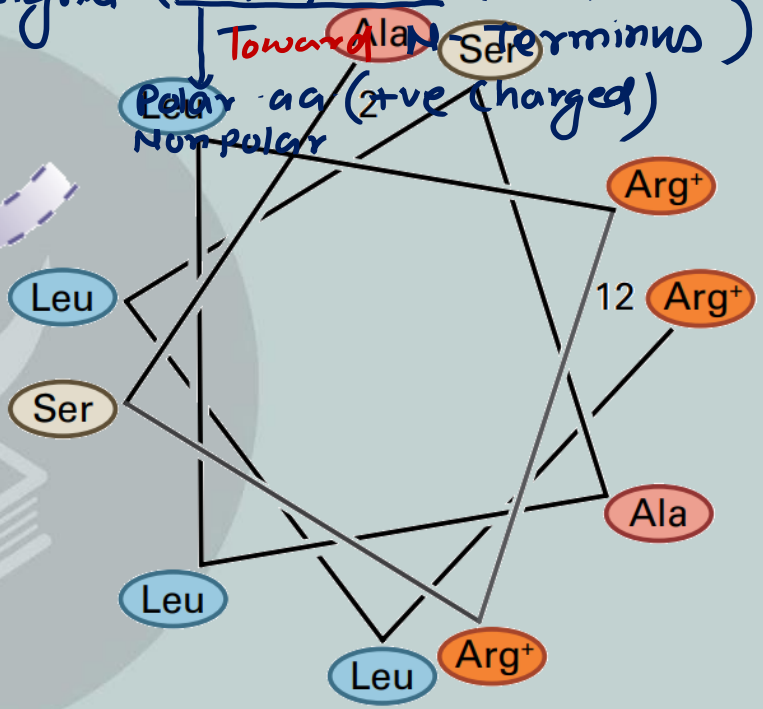
TIM = Translocator of the Inner Mitochondrial membrane;

TOM = Translocator of the Outer Membrane

Presequence → matrix Targeting Signal (Amphipathic α Helix)



Role in Protein Targeting.



Mitochondrial Protein targeting

Require membrane potential

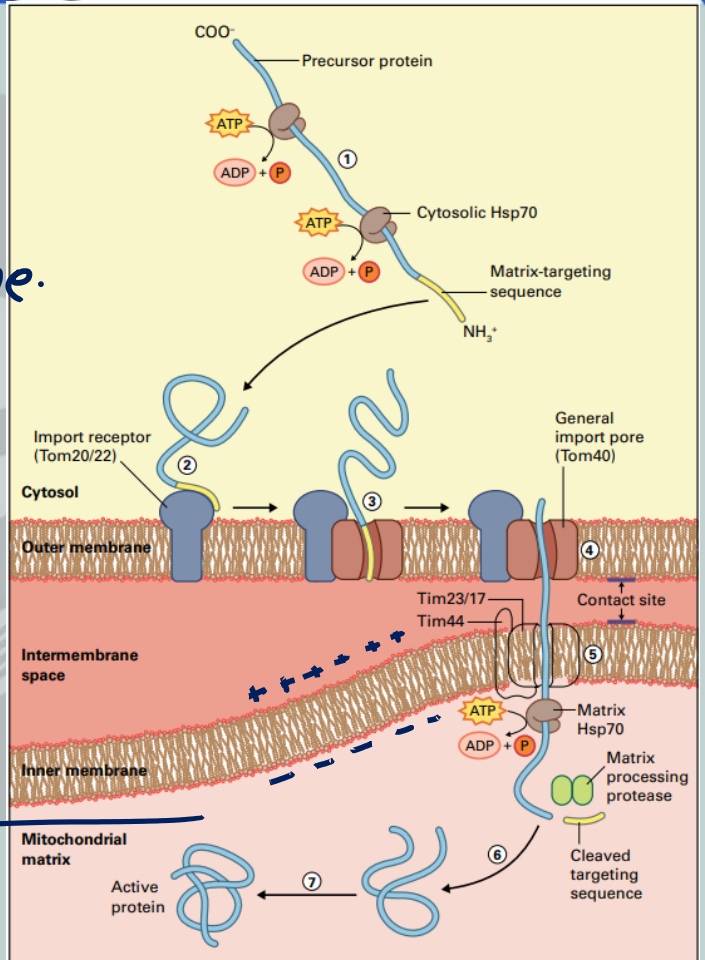
- Across inner mitochondrial membrane.

MTS = Matrix Targeting Signal.

* if Electric potential is disturbed
  ↓
  Matrix Targetting Failed.

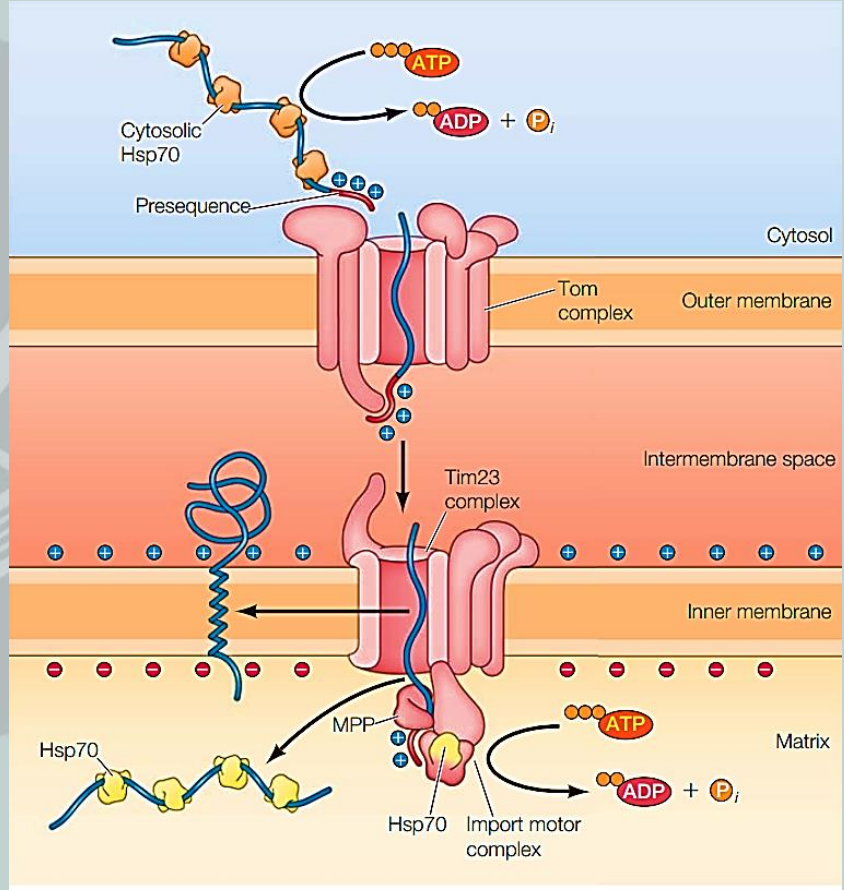
-ve Charged

- Attract +ve charged MTS



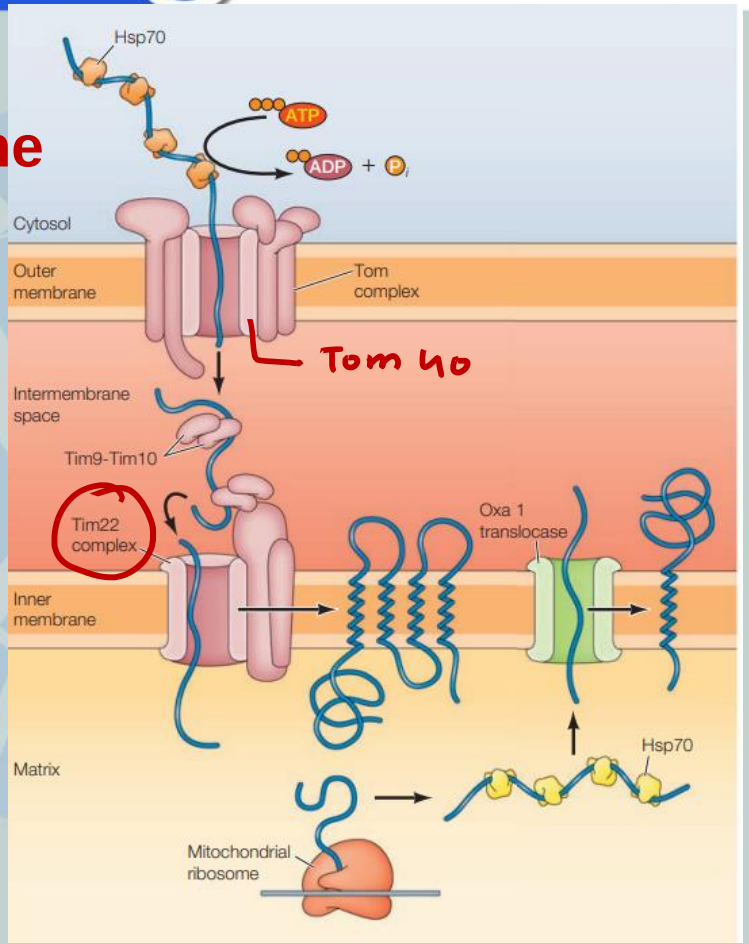
Import of mitochondrial proteins with presequences

- the presequence binds to the Tim23 complex in the inner membrane.
- In the matrix, an import motor complex containing an Hsp70 chaperone uses ATP hydrolysis to drive translocation of the protein across the inner membrane.
- Some proteins with transmembrane domains exit Tim23 laterally into the inner membrane.
- Proteins destined for the mitochondrial matrix associate with soluble Hsp70 chaperones that assist in their folding.
- Presequences are removed by the mitochondrial matrix processing peptidase (MPP).

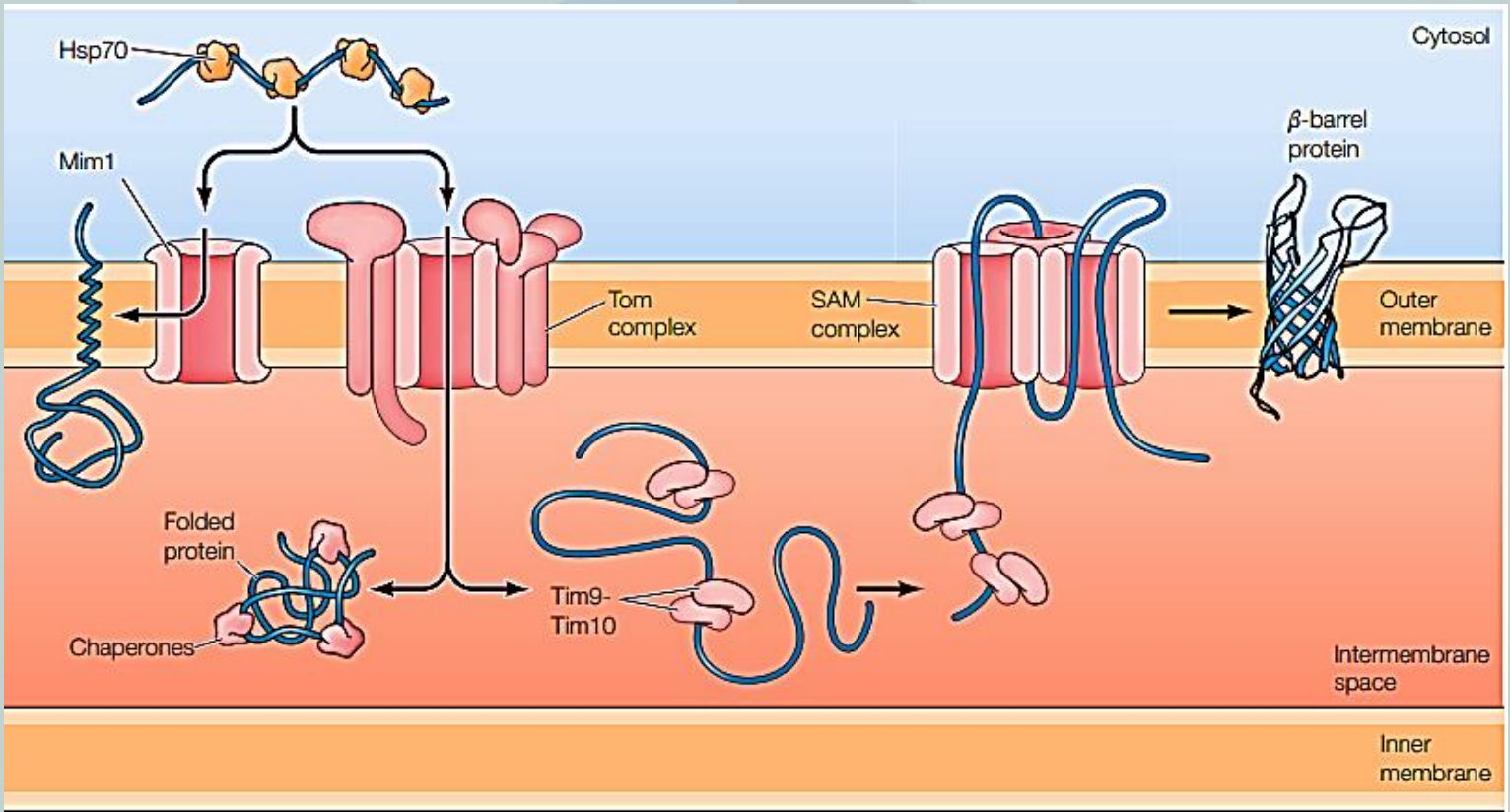


TIM23 complex is bound to a multimeric protein complex containing mitochondrial hsp70, which acts as an import ATPase, using ATP hydrolysis to pull proteins through the pore.

Protein targeting to the mitochondrial inner membrane



Sorting of proteins to the outer membrane and intermembrane space



Functions of mitochondria

matrix

Metabolism

- Oxidative Cycle (in aerobic condition)
- TCA
- Beta oxidation
- Urea Cycle
- Gluconeogenesis (1 - step)
- Formation of ketone body

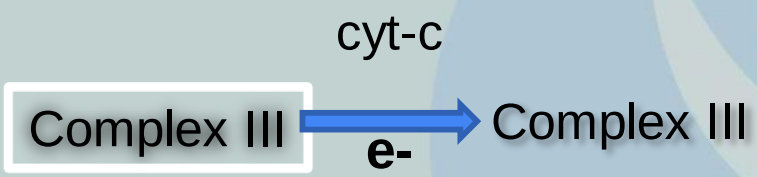
inner mitochondrial membrane – selectively permeable

- Contain almost 70% protein
- ETC complex

Transporters

- ADP/ATP exchanger
- Fo- F1 particle
- iP – Pyruvate transporter
- Glutamate/ aspartate transporter
- Ornithine / citruline transporter

- **Cyt – c** - Peripheral protein of inner membrane present toward inner membranous space



if cyt-c leaks out in the cytosol – signal for intrinsic apoptotic pathways

Mitochondria outer membrane

Elongation of fatty acyl chain

-mainly occurs on SER membrane but also can be seen on Outer mitochondrial membrane

Contain Porin – so that mitochondrial inner membrane can not act as permeability barrier

Disease associated with mitochondria

1- Myoclonic Epilepsy and Red Ragged Fibre

Heart **Brain** **Muscle**

Affected due to non functional

2- Labers optic Neuropathy (LON)

- Due to deffect n Mitochondria

Heredity - maternal

Eg. LON male × Normal female

Normal

Normal male × LON female

LON

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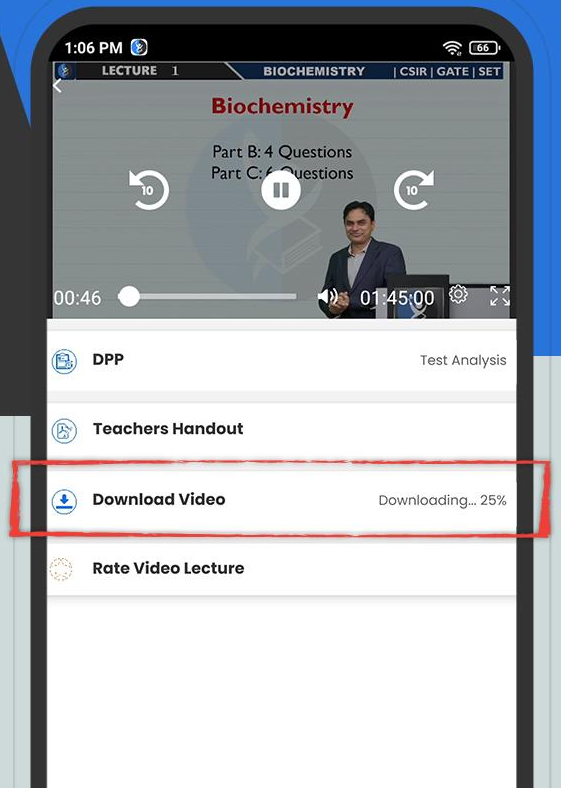

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Share your referral code or Link with your friends.

Step – 02 Earn Cashback

On Every Purchase made using your referral code, you earn 5% instant cashback in your bank account.

Step – 03 Refer More, Earn More

Keep referring as many friends as you can and keep **Earning** cash as much as you can

Friendship is Sharing, Caring, Giving & Helping Each Other...!